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Purification and characterization of a dimethylsulfoniopropionate cleaving enzyme from *Desulfovibrio acrylicus*

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Abstract

An enzyme that cleaves the algal osmolyte dimethylsulfoniopropionate to dimethylsulfide and acrylate was purified almost 400-fold from the marine sulfate- and acrylate-reducing bacterium *Desulfovibrio acrylicus* DSM 10141. Dimethylsulfoniopropionate lyase activity was induced by acrylate and dimethylsulfoniopropionate. At 30°C, the enzyme had a K_m for dimethylsulfoniopropionate of 0.45 mM and a V_{max} of 2590 μmol min^{-1} mg $protein^{-1}$. Dimethylsulfoniopropionate was the only substrate of the enzyme. Among the compounds tested, dimethylsulfoniobutyrate was the most potent inhibitor (K_i=0.25 mM). On a denaturing polyacrylamide gel, the protein migrated as a single band of 49 kDa.

Keywords: Dimethylsulfoniopropionate; Dimethylsulfide; Dimethylpropiothetin dethiomethylase (EC 4.4.1.3); *Desulfovibrio acrylicus*

1. Introduction

Dimethylsulfide (DMS) is one of the most abundant volatile sulfur compounds in the marine ecosystem and is thought to play an important role in the natural sulfur cycle [1]. It has been suggested that DMS also plays a role in the formation of cloud condensation nuclei and thus may be involved in climate regulation [2]. The main precursor of DMS in the marine environment is dimethylsulfoniopropionate (DMSP), an osmolyte produced by a variety of marine algae [3]. DMSP can be degraded by demethylation to 3-mercaptopropionate with 3-*S*-methyl-mercaptopropionate as a possible intermediate, or via cleavage to DMS and acrylate [4,5]. Certain macro- and microalgae [6,7], a variety of aerobic bacteria [8,9], and two anaerobic bacteria, a strain of *Clostridium propionicum* [10] and *Desulfovibrio acrylicus* strain W218 [11], have been found to cleave DMSP to DMS and acrylate. DMSP lyases (dimethylpropiothetin dethiomethylase, EC 4.4.1.3), which catalyze the cleavage of DMSP, have only been purified from the red alga *Polysiphonia paniculata* [6] and the bacterium *Alcaligenes* sp. strain M3A [9]. In this paper we describe the characteristics of a DMSP lyase from *D. acrylicus* strain W218, a marine bacterium that can reduce both sulfate and acrylate.

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2. Materials and methods

2.1. Media, cultivation, and preparation of cell extracts

Desulfovibrio acrylicus strain W218 (DSM 10141) was grown at 30°C in three 20-litre carboys containing sulfide-reduced bicarbonate-buffered mineral medium [11] with 40 mM lactate, 20 mM sulfate, 0.1% yeast extract, and 5 mM DMSP; DMSP was synthesized from DMS and acrylic acid [11]. Twelve hours before harvesting, an additional 3 mM DMSP was added. The following steps were performed under oxic conditions. Cells were harvested by filtration and centrifugation [12]. Cells were washed twice in 25 mM potassium phosphate buffer (pH 7.1) containing 25.0 g l^{-1} NaCl and 3.0 g l^{-1} $MgCl_2 \cdot 6H_2O$. Cell-free extract was prepared by suspending the cell paste in an equal volume of 25 mM potassium phosphate buffer (pH 7.1) and disrupting the cells by three passages through a French pressure cell (106 MPa), followed by centrifugation for 30 min at $39\,000 \times g$.

2.2. Purification of DMSP lyase

Ammonium sulfate was added to 60% saturation to the supernatant obtained after ultracentrifugation of the cell-free extract (1 h at $100\,000 \times g$). This mixture was stirred on ice for 30 min and then centrifuged at $10\,000 \times g$ for 20 min. The supernatant was loaded onto a Phenyl Sepharose 6 Fast Flow high sub (Pharmacia) column (27×2.6 cm), which had been equilibrated with 20 mM potassium phosphate buffer (pH 7.1) containing 60% ammonium sulfate. After a step elution to 30% ammonium sulfate, the enzyme was eluted using a 30–0% ammonium sulfate gradient (1.5 ml min^{-1}; 100 ml). Fractions containing DMSP lyase activity were pooled (24 ml) and, after overnight dialysis (10 kDa cut off size) against a 20 mM Tris-HCl buffer (pH 9.2) at 4°C, loaded onto a Q-Sepharose HP (Pharmacia) column (6×0.6 cm), which had been equilibrated with a 20 mM Tris-HCl (pH 9.0) buffer. The column was eluted with a linear gradient of 0–1 M NaCl (0.8 ml min^{-1}; 24 ml). The active fractions were pooled (2 ml) and loaded onto a Superdex 200 (Pharmacia) column (XK 16/60), which had been equilibrated with a 20 mM Tris-HCl buffer (pH 7.1) containing 0.15 M NaCl and eluted with the same buffer (1 ml min^{-1}; 240 ml).

2.3. Determination of enzyme activity and characteristics

DMSP lyase was assayed spectrophotometrically by following the formation of acrylate from 5 mM DMSP at 220 nm [11]; one unit of enzyme activity (U) produces 1 μmol of acrylate min^{-1}. The pH optimum was determined in 25 mM potassium phosphate buffer (pH 6.0 to pH 8.3) or 25 mM Tris-HCl buffer (pH 7.3 to pH 9.2). The temperature optimum was determined between 13°C and 44°C. Activity was measured after 5 min preincubation at the desired pH or temperature. Diethylsulfoniopropionate was a generous gift of Dr. B.F. Taylor (University of Miami, USA). Dimethylsulfoniobutyrate and dimethylsulfoniopentanoate were a generous gift of Dr. D. Russell (University of Southampton, UK).

Table 1
DMSP lyase activities in cell-free extracts of *Desulfovibrio acrylicus* strain W218

Compound	DMSP lyase activity[a] (U mg protein $^{-1}$)	% of DMSP-induced activity
Sulfate[b]	0.02	0.2
Acrylate[c]	14.2	151
DMSP[b]	9.4	100
Dimethylsulfoxide[b]	0.4	4
Dimethylsulfide[b]	0.3	3
Acrylamide[b]	0.6	6

[a]The data represent the averages of three individual cell extracts.
[b]Cells grown on 20 mM lactate, 0.01% yeast extract, and 20 mM sulfate; potential inducers were added to the following concentrations: DMSP, 8 mM in total; dimethylsulfoxide, 5 mM; dimethylsulfide, 5 mM; acrylamide, 2.5 mM.
[c]Cells grown on 20 mM lactate, 0.01% yeast extract and 20 mM acrylate.

Table 2
Purification of DMSP lyase from *Desulfovibrio acrylicus* strain W218

Step	Total protein (mg)	Total activity (U)	Yield (%)	Specific activity (U mg^{-1})	Purification (fold)
Crude extract	775.0	4170	100	5.4	1
Uc[a]/60% $(NH_4)_2SO_4$	110.7	3740	90	33.8	6.3
Phenyl-Sepharose	28.6	2160	52	75.5	14
Q-Sepharose	1.5	1140	29	760	141
Superdex 200	0.14	294	7	2100	389

[a]Ultracentrifugation.

2.4. SDS-PAGE and protein determination

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the following prestained molecular mass standards (Pharmacia) were used: phosphorylase B (106 kDa), bovine serum albumin (80 kDa), ovalbumin (49.5 kDa), carbonic anhydrase (32.5 kDa), soybean trypsin inhibitor (27.5 kDa), and lysozyme (18.5 kDa). Protein was determined according to Bradford with bovine serum albumin as a standard. The protein concentration of the purified enzyme preparation was determined from the absorbance difference between 280 and 235 nm according to [13].

3. Results and discussion

3.1. Induction of DMSP lyase activity

The DMSP lyase of *D. acrylicus* strain W218 is an inducible enzyme. In the absence of DMSP or acrylate only a low activity was found in cell-free extracts (Table 1). Cells grown on lactate with 20 mM acrylate as electron acceptor had the highest activity (14.2 U mg protein^{-1}). Acrylamide, which was shown to be an inducer of the DMSP lyase of *Alcaligenes* sp. strain M3A [9], but not of the enzyme in *Pseudomonas doudoroffii* [14], did not induce the lyase in *D. acrylicus*. Neither did DMS nor dimethylsulfoxide. The latter did induce DMSP lyase activity in strain M3A and in *P. doudoroffii* [14].

3.2. Purification and enzyme characteristics

Anoxically prepared cell-free extracts had similar DMSP lyase activities as oxically prepared extracts. Therefore, the purification of the enzyme was performed under oxic conditions. DMSP lyase of *D. acrylicus* was purified 389-fold to a specific activity of 2100 U mg protein^{-1} (Table 2). The purified protein showed one distinct band after SDS-PAGE corresponding to an M_r of approximately 49 kDa (Fig. 1). The native M_r could not be determined using gel filtration chromatography because of interaction of the protein with the column material; gel filtration over Superdex 200 was however very useful in the purification procedure. The DMSP lyase from *Alca-*

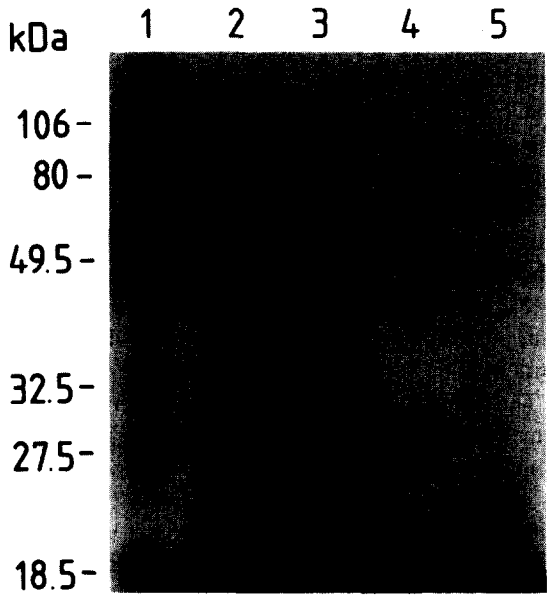

Fig. 1. SDS-polyacrylamide gel (10%) showing the different steps in the purification of the DMSP lyase of *Desulfovibrio acrylicus*. Lanes: 1, molecular mass markers (6 μg); 2, cell-free extract (20 μg); 3, Phenyl Sepharose (10 μg); 4, Q-Sepharose (6 μg); 5, Superdex 200 (5 μg). The gel was stained with 0.1% Coomassie brilliant blue R250.

Table 3
Inhibition of DMSP lyase by different compounds

Compound	Inhibition[a] (%)
Glutaraldehyde (0.5%)	90
Sodium dodecyl sulfate (0.1%)	99
Chloroform (1 mM)	0
3-*S*-Methylmercaptopropionate (2.5 mM)	0
Dimethylsulfoxide (2.5 mM)	21
3-Mercaptopropionate (2.5 mM)	18
Betaine (2.5 mM)[b]	23
Diethylsulfoniopropionate (2.55 mM)[c]	47
Dimethylsulfoniobutyrate (0.255 mM)[d]	51
Dimethylsulfoniopentanoate (2.55 mM)[e]	48

[a]This value represents the average of three measurements.
[b]$K_i = 7.1$ mM.
[c]$K_i = 1.1$ mM.
[d]$K_i = 0.25$ mM.
[e]$K_i = 2.7$ mM.

ligenes sp. strain M3A had a native M_r of 48 kDa and consisted of one subunit [9].

The K_m for DMSP was determined to be 0.45 mM at 30°C and 0.41 mM at 20°C. The V_{max} at 30°C was 2590 U mg protein^{-1} and 1498 U mg protein^{-1} at 20°C. The optimum pH was 8.8 and the temperature at which the highest activity was measured without inactivation of the enzyme was 30°C. The enzyme activity was not inhibited or activated by 100 mM NaCl, as was also shown for the enzyme from *Alcaligenes* sp. strain M3A [9]. The lyase from *P. doudoroffii* was most active at 600 mM NaCl [14]. The K_m of the purified DMSP lyase of *D. acrylicus* was comparable to the K_m value for DMSP (0.4 mM) of whole cells [11]. Cells of *P. doudoroffii* had a far lower K_m for DMSP than the enzyme from the organism, possibly due to the presence of a DMSP binding protein [14].

The DMSP analogues diethylsulfoniopropionate, dimethylsulfoniobutyrate, and dimethylsulfoniopentanoate were not converted by the enzyme but acted as inhibitors of the cleavage of DMSP (Table 3), with dimethylsulfoniobutyrate being the most potent inhibitor ($K_i = 0.25$ mM). Dimethylsulfoxide and 3-mercaptopropionate inhibited the DMSP lyase activity weakly (21 and 18% respectively). *S*-Methylmethionine was neither a substrate nor an inhibitor of the enzyme. Conversely, a partially purified methylmethionine lyase from a *Corynebacterium* strain was not active towards DMSP [15]. Unlike the enzymes from *Alcaligenes* sp. strain M3A and *P. doudoroffii* [14], the DMSP lyase from *D. acrylicus* was not inhibited by 3-*S*-methylmercaptopropionate (Table 3). Glutaraldehyde (0.5% v/v), which is often added to sediment suspensions to inhibit microbial activity, did not inhibit the lyase activity completely (Table 3). This result indicates that the DMSP concentrations which have been measured in sediments using this technique (e.g. [16]) are possibly an underestimation of the actual values.

The DMSP lyase from this obligate anaerobe is rather similar to the enzyme from *Alcaligenes* sp. strain M3A with respect to its behaviour during the purification, its substrate specificity, and polypeptide M_r. The kinetic properties of the *D. acrylicus* enzyme, however, are slightly different (lower K_m and higher V_{max}), and it is more sensitive to glycine betaine and less sensitive to 3-*S*-methylmercaptopropionate (cf. [9,14]).

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